Translation of Referential Document 2

The 106th Annual Meeting of the Japanese Ophthalmological Society

Dates: May 23th, Thursday to 26th, Sunday, 2002

Places: Sendai International Center, Miyagi-ken Sports Center

President: TAMAI Makoto

Host: Japanese Ophthalmological Society

Mr. TANAKA Koichiro

First department of Ophthalmology

Toho University,

Dear Sir,

We appreciate your attending the 106th Annual Meeting of the Japanese Ophthalmological Society held at Sendai.

After fair judgment, the lecture which you reported was selected as excellent lecture for the 106th Annual Meeting of the Japanese Ophthalmological Society. We are pleased to announce the selection result and send you a commemorative gift.

It is the custom that the excellent lecture of the Japanese Ophthalmological Society is exhibited again at the meeting of the Japanese Clinical Ophthalmological Society in the same year. The 56th Annual Meeting of the Japanese Clinical Ophthalmological Society will be held at Morioka, since Thursday, September 26.

Finally, we would wish your further development.

Very truly yours,

June 4, 2002

TAMAI Makoto

Professor of School of Medicine, Tohoku University

President of the 106th Annual Meeting

of the Japanese Ophthalmological Society

The Effects of the Viscoelastic Materials on Bacterial Proliferation and New-quinolone Action

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Original Article

The Effects of the Viscoelastic Materials on Bacterial Proliferation and New-quinolone Action

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ABSTRACT

Purpose : To evaluate the effects of viscoelastic materials on bacterial proliferation and inhibition of newquinolones such as Levofloxacin and Norfloxacin.

Methods : 1) The difference in the bacterial proliferation in the presence of a viscoelastic material was evaluated. 2) Antibacterial drug action in the presence of a viscoelastic material was measured using inhibition circles. Viscoelastic materials were either layered or mixed as a solution.

Results : Viscoelastic materials alone neither promoted nor inhibited bacterial proliferation. In the Layered group, inhibition of antibacterial drug action was observed. In the Mixture group, antibacterial drug action was unaffected.

Conclusion : Viscoelastic material did not influence the bacterial proliferation. The antibacterial drug action beneath the viscoelastic material layer was thus decreased. The decrease did not occur in the Mixture group, suggesting that a mixture of viscoelastic materials and antibacterials might be superior in preventing endophthalmitis.

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KEYWORDS : endophthalmitis, viscoelastic materials, newquinolone

In ophthalmology, postoperative bacterial endophthalmitis is the most serious potential complication[1,2]. Though some patients retain excellent visual acuity due to the widespread use of early-stage intravitreous antibiotic therapy and advances in medication for postoperative bacterial endophthalmitis, visual acuity outcomes remain poor[3-6]. As a result, prevention of bacterial endophthalmitis after intraocular surgery remains highly desirable.

Viscoelastic materials such as sodium hyaluronates and chondroitin sulfates are indispensable in intraocular surgery, especially in modern cataract

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surgery[7,8]. Cohesive-type viscoelastic materials such as sodium hyaluronate help maintain eyeball form in intraocular surgery. Dispersive-type viscoelastic materials, such as chondroitin sulfate, break into small pieces when aspirated, and thus cannot be completely removed from the intraocular space. When the corneal endothelium is covered with dispersive viscoelastic materials, it is protected in cataract surgery[9-13]. However, there are reports of residual viscoelastic material in the intraocular space after intraocular surgery[14,15].

Because the effects of residual viscoelastic materials on bacterial proliferation in the intraocular space are unknown, the purpose of this study was to evaluate the effects of viscoelastic materials on both bacterial proliferation and antibacterial drug action. In addition, we examined combinations of viscoelastic materials and antibacterials from the perspective of postoperative bacterial endophthalmitis prevention.

Methods

1. Effects of viscoelastic materials on bacterial proliferation

Strain: Methicillin-resistant *Staphylococcus aureus* MK99-3 (MRSA MK99-3) and *Stenotrophomonas maltophilia* TK-1 (*S. maltophilia* TK-1) were used. MRSA MK99-3 was obtained from a patient with ocular infection and *S. maltophilia* TK-1 from another patient with postoperative bacterial endophthalmitis.

Viscoelastic materials: Healon® (Pfizer, USA), a hyaluronate sodium solution and Viscoat® (ALCON, USA), a sodium hyaluronate and chondroitin sulfate sodium solution, were used. Healon® contains 10 mg/ml of sodium hyaluronate, 5000 kDa, dissolved in physiological sodium chloride-phosphate buffer (pH 7.0 to 7.5). Viscoat® contains 30 mg/ml of sodium hyaluronate, 500 kDa, and 30 mg/ml of sodium chondroitin sulfate, 22500 Da, dissolved in physiological buffer (pH 7.0 to 7.4). Viscosity (cps) at zero shear rate was 243 for Healon and 58 for Viscoat.

Culture: Bacterial stock solution (about 10^8 CFU/ml, after the cultivation in advance of cryopreservation bacterium) was diluted with physiological saline solution (saline adjustment bacterial liquid, 145 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5.6 mM glucose, 15 mM HEPES-NaOH pH 7.4) or Müeller-Hinton (MH) liquid medium (MH adjustment bacterial liquid), and adjusted to about 10^6 CFU/ml. A penicillin cup (a suspended metal cylinder, 0.3 mm^2 area at the base and 10.0 mm in height) was used for bacterial culture. To monitor the bacterial growth in Müeller-Hinton broth the authentic method recommended by the Japanese Society of Chemotherapy was nsed. The composition of Müeller-Hinton liquid medium (g/liter) was meat infusion 2.0; casein hydrolysate 17.5; starch 1.5 pH: 7.3±0.1 at 25℃.

Cultures were divided into the following four groups: A) Cultures with 0.1 ml viscoelastic materials and 0.1 ml saline adjustment bacterial liquid (viscoelastic materials+saline group). B) Cultures with 0.1 ml viscoelastic materials and 0.1 ml MH adjustment bacterial liquid (viscoelastic materials+MH liquid medium group). C) Cultures with only 0.1 ml saline adjustment bacterial liquid (saline group). D) Cultures with only 0.1 ml MH adjustment bacterial liquid (MH liquid medium group).

Eight specimens were prepared for each group (32 specimens with Healon and 32 specimens with Viscoat). All specimens were cultured in an incubator at 35 degrees.

Viable count: The viable count of specimens was measured after 2, 4, 6 and 20 hrs. of bacterial culture. Two specimens from each group were examined at each time point. The two-way layout analysis of variance method (ANOVA) was used for statistical analyses between the proliferation profiles of each group. To plot the proliferation profile, the average of two specimens was assumed to be the viable count of each group.

2. Effects of viscoelastic materials on antibacterial drug action

Viscoelastic materials: Healon® and Viscoat® were used.

Antibacterials: 0.5% Levofloxacin (LVFX, Cravid®, Santen Pharmaceutical, JAPAN) and 0.3% Norfloxacin (NFLX, NOFLO®, Banyu Pharmaceutical, JAPAN) were used. The highest concentration in the aqueous humor (AQCmax) of 0.5% LVFX is 2.17 μg/ml[16]. According to their articles, 50 ml of newquinolones was instilled into the cul-de-sac of New Zealand White rabbit eyes 3 times at 15-minute

intervals and the drug concentrations in the aqueous humor were examined by high performance liquid chromatography. The AQCmax was calculated using the one-compartment method.

Based on AQCmax of 0.5% LVFX, each antibacterial was adjusted to either 10.0 μg/ml, 5.0 μg/ml, 2.5 μg/ml, 1.25 μg/ml, or 0.625 μg/ml.

Authorization strain: *Bacillus subtilis* ATCC6633 (*B. subtilis* ATCC6633) was used. This strain is a common authorization strain in Japan.

Culture: 0.1 ml of the authorization bacterial liquid (1.4×10^8 CFU/ml) was spread on a sterile 4 % Heart Infusion agar and the penicillin cup was put on the surface. Cultures were divided into the following four groups. A) Cultures with penicillin cups injected with only 0.1 ml antibacterials (Antibacterial group). The concentration of antibacterials was 10.0 μg/ml, 5.0 μg/ml, 2.5 μg/ml, 1.25 μg/ml or 0.625 μg/ml. B) Cultures with penicillin cups injected with only 0.1 ml viscoelastic materials (Viscoelastic material group). C) Cultures with penicillin cups injected with 0.1 ml antibacterials after injection of 0.1 ml viscoelastic materials (Layered group). First, viscoelastic material was injected to form the layer. Next, the antibacterial was injected over the viscoelastic material. The concentration of antibacterial was either 5.0 μg/ml or 2.5 μg/ml. D) Cultures with penicillin cups injected with 0.1 ml viscoelastic materials and 0.1 ml antibacterials (Mixture group). Before injection, the viscoelastic materials and antibacterials were thoroughly mixed in a glass syringe until uniform. The concentration of antibacterial drug was either 5.0 μg/ml or 2.5 μg/ml.

Two specimens were prepared for each group. The specimens were cultured for 24 hours in an incubator at 35 degrees.

Inhibition zone measurement: The inhibition zone's longest axis was measured after 24 hours of bacterial culture. The standard concentration curve for each antibacterial drug was determined using the average inhibition zone diameter of the Antibacterial group. The one-way layout ANOVA was used for statistical analyses.

Results

1. Effects of viscoelastic materials on bacterial proliferation

The proliferation profile of MRSA MK99-3 with Healon is shown (Fig. 1). The extent of bacterial proliferation in the MH liquid medium group was greater than that of the Saline group ($p<0.01$). The extent of bacterial proliferation in the Viscoelastic materials+MH liquid medium group was greater than that of the Viscoelastic materials+saline group ($p<0.01$). No significant difference was observed in the extent of bacterial proliferation between the Viscoelastic materials+MH liquid medium group and the MH liquid medium group, or between the Viscoelastic materials+saline group and the Saline group.

The proliferation profile of *S. maltophilia* TK-1 with Healon is shown (Fig. 2). The extent of bacterial proliferation in the MH liquid medium group was greater than that of the Saline group ($p<0.01$). The extent of bacterial proliferation in the Viscoelastic materials+MH liquid medium group was greater than that of the Viscoelastic materials+saline group ($p<0.01$). No significant difference was observed in the extent of bacterial proliferation between the Viscoelastic materials+MH liquid medium group and the MH liquid medium group, or between the Viscoelastic materials+saline group and the Saline group.

The two viscoelastic materials, Healon and Viscoat, exhibited similar characteristics with both bacteria.

2. Effects of viscoelastic materials on antibacterial drug action

In the Viscoelastic material group, neither Healon nor Viscoat formed an inhibition zone.

The relation between LVFX concentration and inhibition zone diameter is shown (Fig. 3). The reference LVFX concentration curve obtained from the results of the LVFX Antibacterial group is also shown (LVFX reference trendline, coefficients of determination: $R2=0.9812$). In the LVFX Layered group, the inhibition zone diameters were shorter as compared with the LVFX reference trendline ($p<0.01$), particularly in the LVFX Viscoat Layered group; the LVFX Viscoat and Healon Layered groups significantly differed ($p<0.01$). In the

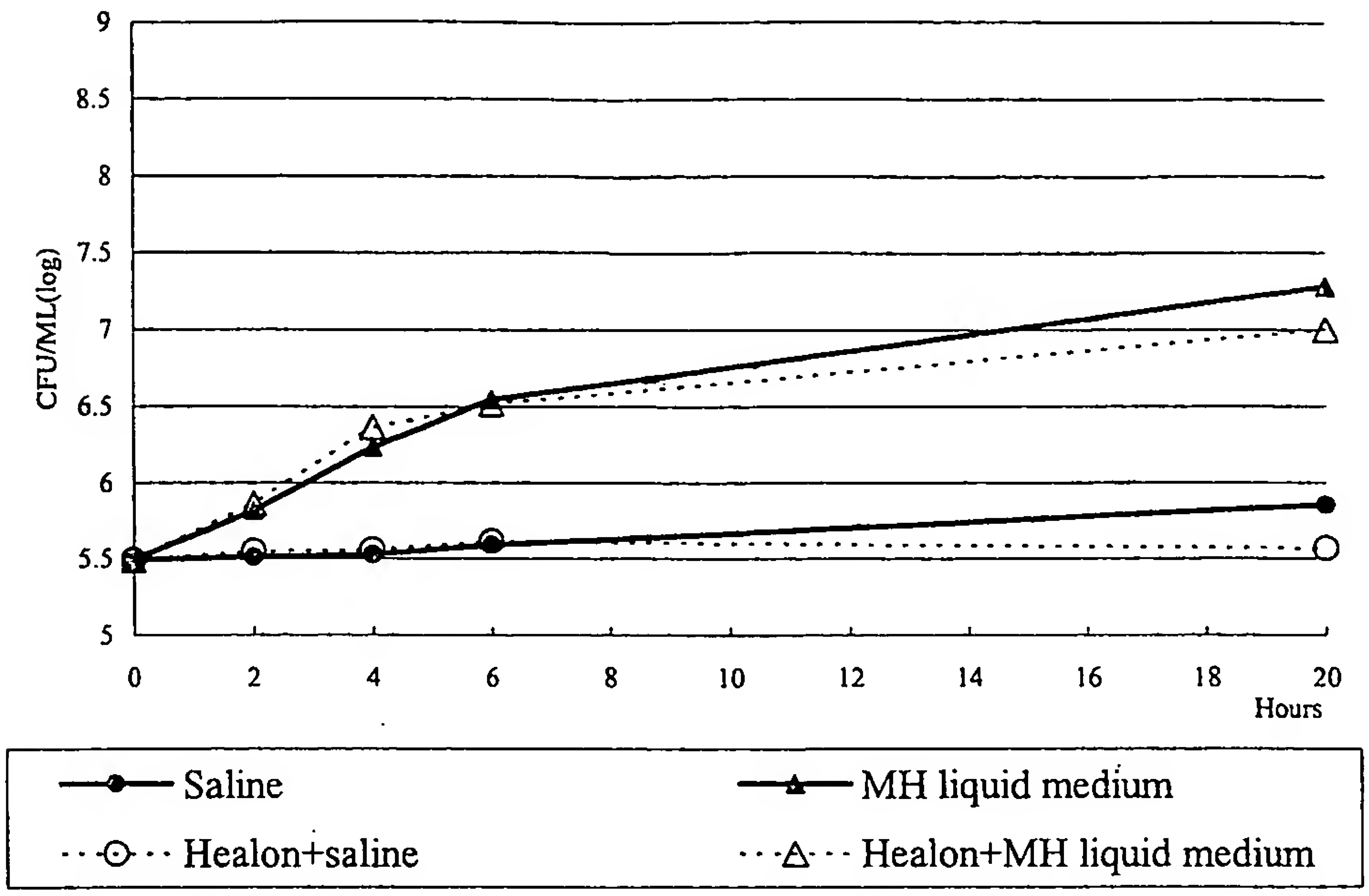

Fig. 1 Proliferation curves of Methicillin-resistant *Staphylococcus aureus* MK99-3 with and without Healon
*Healon+MH liquid medium vs. Healon+saline, *MH liquid medium vs. saline,
*Healon+MH liquid medium vs. saline, *Healon+saline vs. MH liquid medium
(*$p<0.01$)
Healon neither promoted nor inhibited MRSA MK99-3 proliferation.

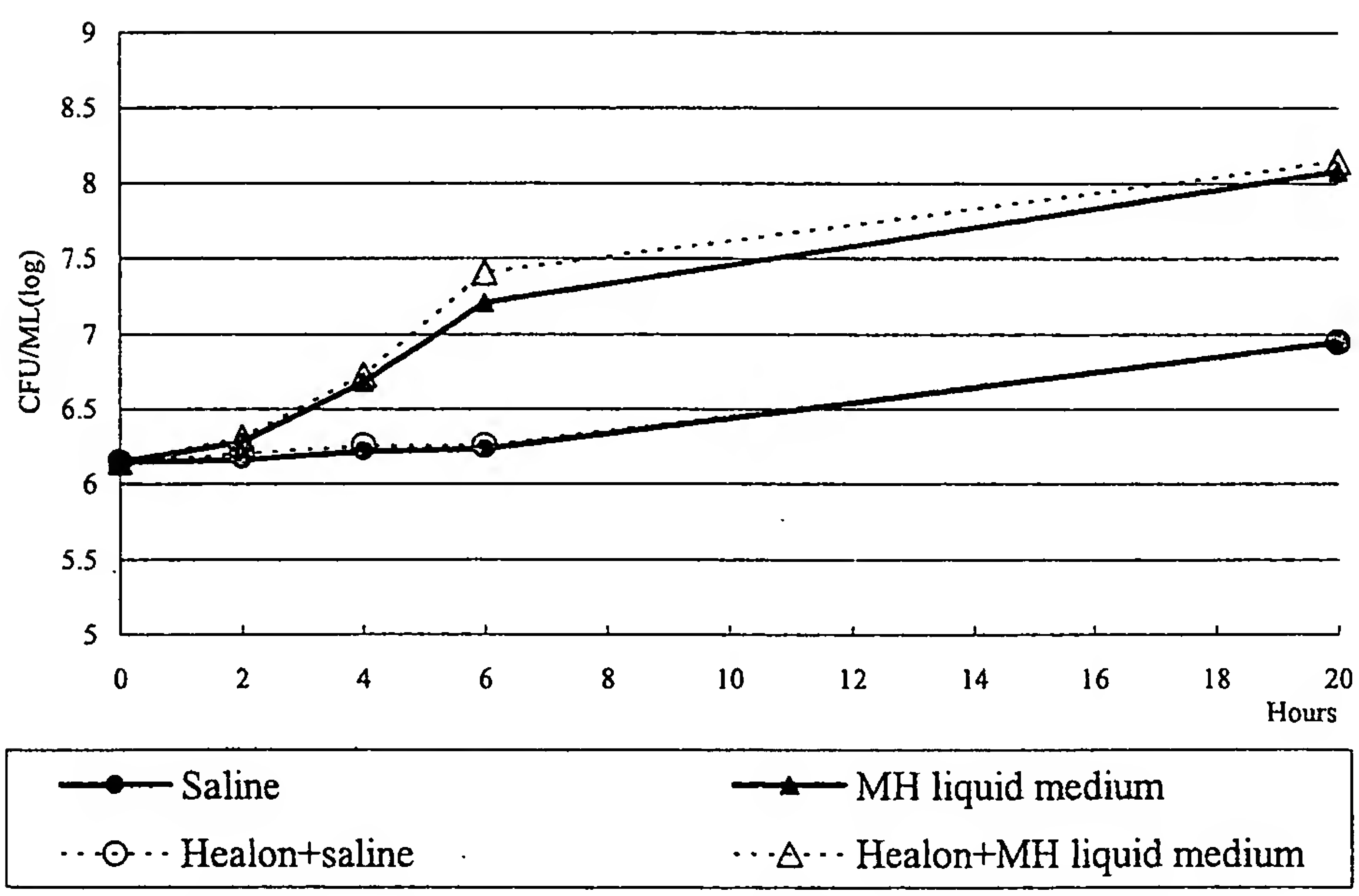

Fig. 2 Proliferation curves of *Stenotrophomonas maltophilia* TK-1 with and without Healon
*Healon+MH liquid medium vs. Healon+saline, *MH liquid medium vs. saline,
*Healon+MH liquid medium vs. saline, *Healon+saline vs. MH liquid medium
(*$p<0.01$)
Healon neither promoted nor inhibited *S. maltophilia* TK-1 proliferation.

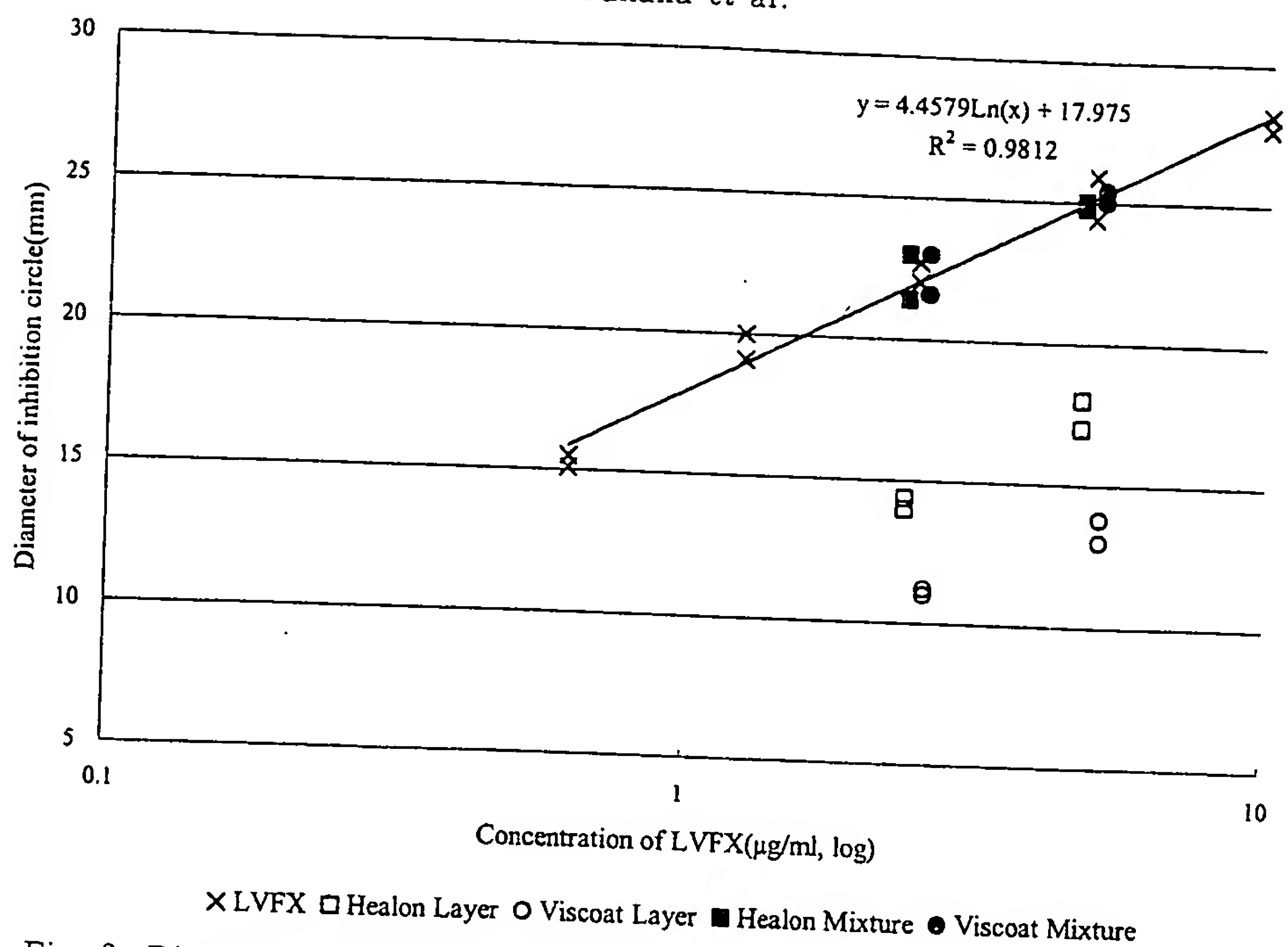

Fig. 3 Diameter of inhibition circle by Levofloxacin concentration
*LVFX reference trendline vs. Healon Layer, *LVFX reference trendline vs. Viscoat Layer
*Healon Layer vs. Healon Mixture, *Viscoat Layer vs. Viscoat Mixture,
*Healon Layer vs. Viscoat Layer
(*$p<0.01$)
In the LVFX Layer groups, decreased inhibition circle diameters were noted. The decrease in the LVFX Layer group of Viscoat was remarkable.
In the LVFX Mixture groups, the Healon and Viscoat inhibition circle diameters were nearly identical to the LVFX reference trendline.

LVFX Mixture group, the Healon and Viscoat inhibition zone diameters were nearly identical to the LVFX reference trendline ($p>0.05$). Comparing the inhibition zone diameters of the LVFX Layered group with the LVFX Mixture group, significant differences were noted with both viscoelastic materials ($p<0.01$).

The relation between NFLX concentration and inhibition zone diameter is shown (Fig. 4). The reference NFLX concentration curve obtained from the results of the NFLX Antibacterial group is also shown (NFLX reference trendline, coefficients of determination: $R2=0.9859$). In the NFLX Layered group, shorter inhibition zone diameters were noted as compared with the NFLX reference trendline ($p<0.01$), particularly in the NFLX Viscoat Layered group; the difference between the NFLX Viscoat and Healon Layered groups significantly differed ($p<0.01$). In the NFLX Mixture groups, the Healon and Viscoat mixture groups' inhibition zone diameters were nearly identical to the NFLX reference trendline ($p>0.05$). In a comparison of the inhibition zone diameters of the NFLX Layered group and the NFLX Mixture group, significant differences were existed with both viscoelastic materials ($p<0.01$).

Discussion

The relationship between viscoelastic materials and ocular infection is important because, although viscoelastic materials are manufactured under sterile conditions, an outbreak of postoperative endophthalmitis due to contaminated viscoelastic materials has been reported[17]. The time point of contamination is uncertain, but the possibility of contamination is incontrovertible. One report found evidence of *Staphylococcus epidermidis* proliferation in viscoelastic material[18]. However, Healon and

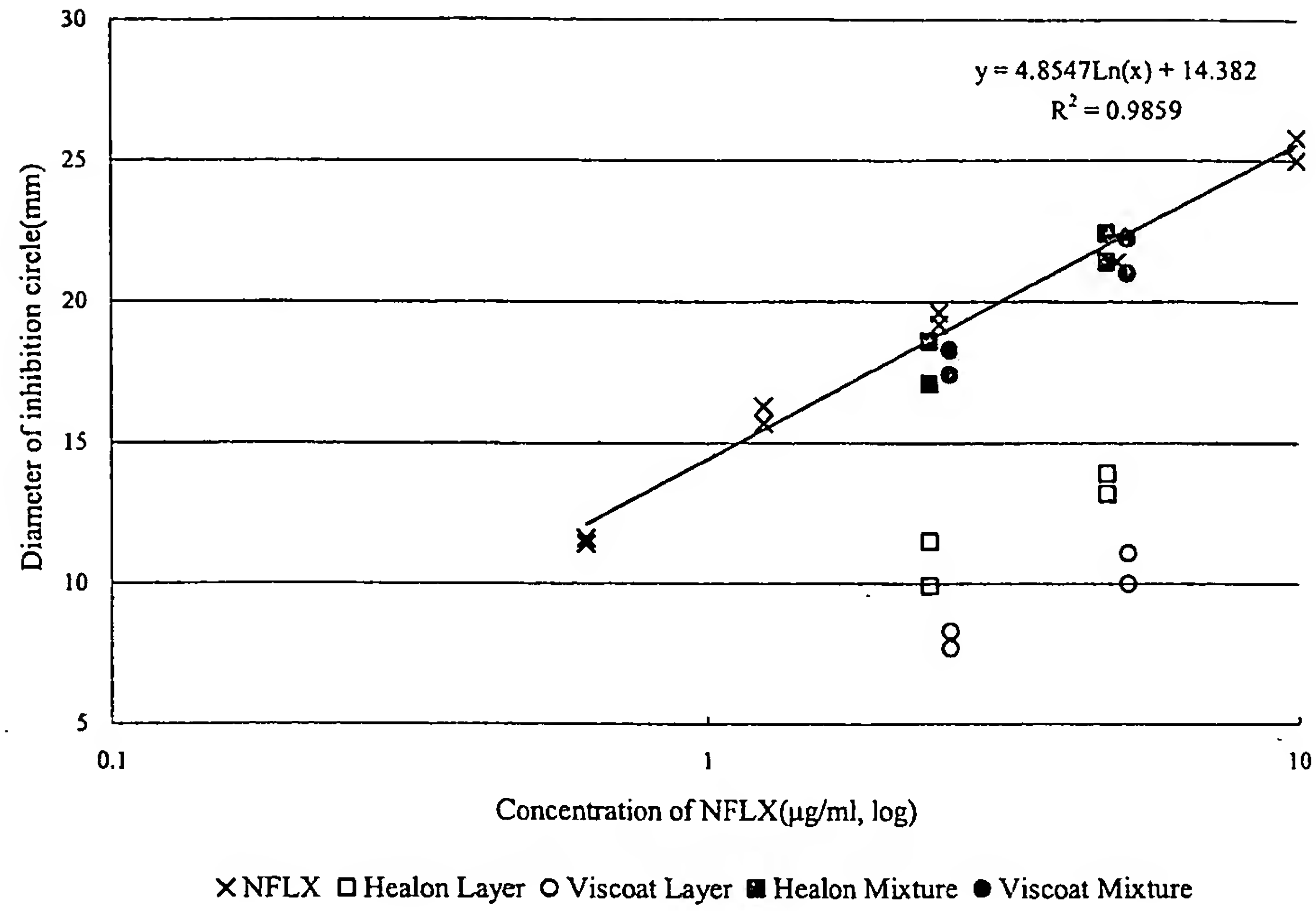

Fig. 4 Diameter of inhibition circle by Norfloxacin concentration
*NFLX reference trendline vs. Healon Layer, *NFLX reference trendline vs. Viscoat Layer
*Healon Layer vs. Healon Mixture, *Viscoat Layer vs. Viscoat Mixture, *Healon Layer vs. Viscoat Layer
(*$p<0.01$)
In the NFLX Layer groups, decreased inhibition circle diameters were noted as compared with the NFLX reference trendline. The decrease in the NFLX Layer group of Viscoat was remarkable.
In the NFLX Mixture groups, the Healon and Viscoat inhibition circle diameters were nearly identical to the NFLX reference trendline.

Viscoat alone neither promoted nor inhibited the proliferation of MRSA MK99-3 and *S. maltophilia* TK-1 and had no antibacterial action against *B. subtilis* ATCC6633 in the present study. Bacterial proliferation can be extremely rapid in a nutrient-rich environment, though these conditions are rarely present. However, viscoelastic materials can be contaminated by bacteria such as *S. maltophilia* which are capable of proliferating in nutrient-deprived environments. Contamination by *Pseudomonas aeruginosa* in a nutrient-deprived environment has been observed[19-21]. Moreover, it is obvious that the aqueous humor does present a nutrient-rich environment for bacteria.

The relationship between viscoelastic materials and antibacterials is another important matter which requires further clarification, because they are commonly used together. The presence of a layer of viscoelastic material decreased the antibacterial drug action in the present study. In the Layered group, inhibition of the antibacterial drug action of LVFX and NFLX was observed with Healon and, to a greater extent, with Viscoat. However, in the Mixture group, the antibacterial drug action of LVFX and NFLX was maintained in the presence of Healon and Viscoat. In particular, the decrease in inhibition circle diameter was greater with Viscoat than Healon. Differences in concentrations, molecular weights and the viscosity of viscoelastic materials are believed to explain variations in viscoelastic material action[9-13]. There is a clear difference in the cohesiveness of viscoelastic materials, and this difference explains why the corneal endothelium is better protected by dispersive viscoelastic materials like Viscoat. The fact that Viscoat is not easily washed off by the flow of water might influence its effect on drug activity.

Mixtures of viscoelastic materials and antibacterials

preserved antibacterial drug effect in the present study. However, the layer of the viscoelastic material controlled the antibacterial drug effect. We hypothesize that the layer of viscoelastic material functions as a pharmacokinetic barrier to antibacterial drug penetration; viscoelastic materials do not inhibit antibacterial drug effect, they delay drug penetration. This delay in antibacterial drug penetration caused by viscoelastic materials might present an increased risk of bacterial endophthalmitis. Viscoelastic materials used for intraocular surgery cannot be completely removed from the intraocular space[14,15]. Furthermore, dispersive viscoelastic materials, which can remain in the intraocular space, are now being used[9,22]. Numerous surgeons have reported that bacteria spread to the aqueous humor can occur during cataract surgery, even when all possible preventive measures are taken[23-32], suggesting that residual viscoelastic materials may promote bacterial infection.

The development of measures to combat endophthalmitis resulting from residual viscoelastic materials depends on modifying viscoelastic materials. Perhaps viscoelastic materials can prevent endophthalmitis if supplemented by antibacterial drug action. Indeed, we showed that a mixture of viscoelastic materials and antibacterials had no effect on antibacterial drug penetration in the present study. We have labeled this mixture of viscoelastic material and antibacterial "Antibacterial Visco". Indeed, injection of such a mixture into the vitreous body after an outbreak of postoperative bacterial endophthalmitis has been reported[33,34]. However, treatment after endophthalmitis onset is not desirable because it is too late to improve visual acuity in most patients. Prevention remains the best strategy against postoperative bacterial endophthalmitis. Using "Antibacterial Visco" for standard intraocular surgery might aid in prevention of postoperative bacterial endophthalmitis. Moreover, controlled release from "Antibacterial Visco" may be useful in administering antibacterials to the eyeball.

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(J) : in Japanese

細菌増殖およびニューキノロン薬に対する粘弾性物質の影響

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要約

目的：細菌増殖に対する粘弾性物質の影響およびレボフロキサシンやノフロキサシンなどのニューキノロン薬に対する粘弾性物質の影響を評価した。

方法：1）粘弾性物質の有無での細菌増殖の差異を検討した。2）阻止円を用い，粘弾性物質によるニューキノロン薬への影響を調べた。この際，粘弾性物質とニューキノロン薬を混合した状態での影響および粘弾性物質の層による影響の双方を検討した。

結果：粘弾性物質は細菌増殖に対して影響を及ぼさなかった。粘弾性物質の層はニューキノロン薬の阻害をもたらしたが，粘弾性物質とニューキノロン薬を混合した状態では阻害はみられなかった。

結論：粘弾性物質自体では細菌増殖に影響を及ぼさなかった。粘弾性物質の層はニューキノロン薬を阻害したが，混合時にこの阻害がみられなかったことから，粘弾性物質とニューキノロン薬の合剤が眼内炎予防に有用である可能性がある。

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索引用語：眼内炎，粘弾性物質，ニューキノロン

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EXHIBIT C

Incidence and Prevention of Bacterial Endophthalmitis With the Use of Viscoelastic Materials and Newquinolone

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Original Article

Incidence and Prevention of Bacterial Endophthalmitis With the Use of Viscoelastic Materials and Newquinolone

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ABSTRACT

Purpose: We previously reported a delay in newquinolone penetration with viscoelastic materials *in vitro*. In the present study, we attempted to determine the effect of viscoelastic materials on bacterial endophthalmitis and to evaluate "Antibacterial Visco", a novel mixture of viscoelastic material and levofloxacin.

Methods: 1) We developed an endophthalmitis model utilizing anterior chamber inoculation of methicillin-resistant *Staphylococcus aureus* (MRSA) in rabbit. 2) Three groups were then formed to determine the effect of viscoelastic materials on endophthalmitis. A) Mixed inoculation group: inoculation of a mixture of viscoelastic materials and MRSA; B) Separate inoculation group: inoculation of viscoelastic materials followed by inoculation of MRSA; and C) Bacteria inoculation group: inoculation of MRSA. 3) Finally, the effects of a mixture of viscoelastic materials and levofloxacin on endophthalmitis were evaluated; A) antibacterial visco group, B) an eye drop treatment group, C) a non-treatment group, and D) a bacteria inoculation group.

Results: 1) Endophthalmitis occurred at 10^7 CFU/eye, but not at 10^3 CFU/eye. 2) In the Mixed inoculation group, endophthalmitis occurred at 10^3 CFU/eye. No endophthalmitis occurred in the Separate inoculation group or Bacteria inoculation group. 3) Endophthalmitis was able to be prevented in the antibacterial visco group. However, treatment of endophthalmitis was difficult in the eye drop treatment group.

Conclusion: The viscoelastic material fomented the bacterial endophthalmitis. A mixture of viscoelastic material and levofloxacin is effective on the bacterial endophthalmitis prevention.

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KEYWORDS: endophthalmitis, viscoelastic materials, newquinolone

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Postoperative bacterial endophthalmitis is a serious postoperative complication[1,2], although its frequency is only about 0.07% due to recent advances in surgical methods and antibacterial drugs[3-8]. However, when postoperative bacterial endophthalmitis occurs and bacteria reach the vitreous, the prognosis remains extremely poor[3,9-12].

Viscoelastic materials are commonly used in ophthalmic surgery[13,14], and the use of new dispersive viscoelastic materials, in addition to the current cohesive viscoelastic materials, is becoming more widespread[15-17]. To protect the corneal endothelium, dispersive viscoelastic materials are designed to remain in the intraocular space[18-21]. However, the effect of residual viscoelastic material on the incidence of postoperative bacterial endophthalmitis has not been reported. In the previous study, we found that viscoelastic materials delayed antibacterial drug penetration *in vitro*. For the present study, a rabbit bacterial endophthalmitis model was developed, and the effect of viscoelastic materials on bacterial endophthalmitis was examined. In addition, we investigated the effectiveness of a compound we have dubbed "Antibacterial Visco", a mixture of viscoelastic material and levofloxacin in preventing bacterial endophthalmitis.

Methods

1. Development of rabbit bacterial endophthalmitis model

A rabbit bacterial endophthalmitis model was developed and changes in intraocular bacterial count, as well as observational and histopathologic findings were examined.

Laboratory animals: Japanese albino rabbits were maintained in accordance with institutional guidelines and the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmologic and Vision Research. The animals were housed in separate cages under a cycle of 12-hour light and 12-hour darkness.

Strain: Methicillin-resistant *Staphylococcus aureus* MK99-3 (MRSA MK99-3) obtained from a patient with ocular infection was used.

Anesthesia: Ketamine hydrochloride (50 mg/ml) and xylazine hydrochloride (20 mg/ml) were used. The ratio was 7 : 1, respectively. Intraperitoneal injection of 4 ml was given as general anesthesia.

Bacterial liquid inoculation: General anesthesia was administered to 20 Japanese albino rabbits. Paracentesis of 0.1ml of the aqueous humor was then performed. After that, 0.1 ml of bacterial liquid was injected into the anterior chambers of the rabbit eyes. The inoculation bacteria count was adjusted to either approximately 10^3 CFU/eye (N=10) or 10^7 CFU/eye (N=10).

Eyes were observed for corneal opacity, ciliary injection, hypopyon and discharge. Eyes with at least three of these conditions were classified as endophthalmitic. The rabbits were euthanized by injection into the cardiac sac of 4 ml of thiopental sodium (25 mg/ml) either 6, 12, 24, 48 or 72 hours after inoculation, and ophthalmectomy was performed. Cultures of the aqueous humor (0.05 ml/eye) and vitreous humor were prepared. Histopathologic specimens were then taken from the removed eyeballs. After fixing the eyeballs in 10% formalin immediately after removal, Giemsa staining and Hematoxylin-Eosin staining were performed.

2. Effects of viscoelastic material on bacterial endophthalmitis

After the rabbit bacterial endophthalmitis model was developed, viscoelastic material was added. To examine the effect of viscoelastic materials, the timing of viscoelastic and bacterial liquid inoculation was varied.

The details of the laboratory animals, the bacterial strain and the anesthesia are given in the previous section.

Viscoelastic materials: Healon® (Pfizer, USA)-a hyaluronate sodium solution, and Viscoat® (ALCON, USA)-a sodium hyaluronate and chondroitin sulfate sodium solution-were used.

Bacterial liquid inoculation: General anesthesia was administered to 88 Japanese albino rabbits. Then, the anterior chambers of the rabbit eyes were inoculated with viscoelastic material and/or bacterial liquid. There were three inoculation groups.

A) Mixed inoculation group: The ratio of viscoelastic material to bacterial liquid was 9 : 1. Paracentesis of 0.1 ml of the aqueous humor was performed. The anterior chamber was inoculated with 0.1 ml of the mixed liquid (N=16; 8 Healon cases and 8 Viscoat cases). The bacteria count was adjusted

to approximately 100 CFU/eye.

B) Separate inoculation group: 0.1 ml of the aqueous humor was removed by paracentesis. Then, 0.09 ml of viscoelastic material was injected into the anterior chamber, followed by a separate 0.01 ml injection of bacterial liquid (N=64; 32 Healon cases and 32 Viscoat cases). Inoculation of bacterial liquid was done immediately, 6, 12, or 24 hrs after inoculation of viscoelastic materials. The bacteria count was adjusted to approximately 100 CFU/eye.

C) Bacteria inoculation group: 0.1 ml of the aqueous humor was removed by paracentesis. Then, 0.1 ml of bacterial liquid was injected into the anterior chamber (N=8). The bacteria count was adjusted to approximately 100 CFU/eye.

Eyes were observed for corneal opacity, ciliary injection, hypopyon and discharge. Eyes with at least three of these conditions were classified as endophthalmitic. Cultures of aqueous humor (0.05 ml/eye) were made either 24 or 48 hrs after initial inoculation. Rabbits were euthanized 48 hrs after inoculation, and ophthalmectomy was performed. Cultures of the aqueous humor (0.05 ml/eye) and vitreous humor were made. Histopathologic specimens were taken from the removed eyeballs. After fixing the eyeballs in 10% formalin immediately after removal, Giemsa and Hematoxylin-Eosin stainings were performed.

3. Prevention of bacterial endophthalmitis with viscoelastic materials and newquinolone

A rabbit bacterial endophthalmitis model was developed. An antibacterial drug was administered to these rabbits by various methods, and the effectiveness was examined.

Details of the laboratory animals, viscoelastic materials and anesthesia used are given in the previous section.

Antibacterial drug: 0.5% Levofloxacin (LVFX, Cravid®, Santen Pharmaceutical, JAPAN) was used. The highest concentration in aqueous humor (AQCmax) of 0.5% LVFX was 2.17 μg/ml[22].

Strain: *Staphylococcus aureus* Smith was used, because it can be treated with antibacterial drugs and is capable of causing endophthalmitis. The minimum inhibitory concentration of LVFX to *Staphylococcus aureus* Smith was 0.25 μg/ml.

Bacterial liquid inoculation: General anesthesia was given to 52 Japanese albino rabbits. Then, bacterial liquid was injected into the anterior chambers of the rabbit eyes. There were 4 inoculation groups.

A) Antibacterial Visco group (N=20, 10 Healon cases and 10 Viscoat cases): the ratio of viscoelastic material to bacterial liquid to LVFX was 9 ml : 0.5 ml : 0.5 ml. The materials were mixed immediately before inoculation. Paracentesis of 0.1 ml of the aqueous humor was performed, and the total inoculant volume was 0.1 ml/eye. The final concentration of bacteria in the mixture was adjusted to 10^4 CFU/eye. The final concentration of LVFX in the mixture was adjusted to 2 μg/ml.

B) Eye Drop Treatment group (N=20, 10 Healon cases and 10 Viscoat cases): the ratio of viscoelastic material to bacterial liquid was 9 ml : 1 ml. The materials were mixed immediately before the inoculation. Paracentesis of 0.1 ml of the aqueous humor was performed, and the total inoculant volume was 0.1 ml/eye. The final concentration of bacteria in the mixture was adjusted to 10^4 CFU/eye. 0.5% LVFX eye drop treatment and injection of 50 μl of LVFX into the cul-de-sacs of rabbit eyes 4 times/day had been performed on the day before the inoculation. Eye drop treatment continued until the final day of the study.

C) Non-Treatment group (N=8, 4 Healon cases and 4 Viscoat cases): the ratio of viscoelastic material to bacterial liquid was 9 : 1. The materials were mixed immediately before the inoculation. Paracentesis of 0.1 ml of the aqueous humor was performed, and the total inoculant volume was 0.1 ml/eye. The final concentration of bacteria in the mixture was adjusted to 10^4 CFU/eye.

D) Bacteria inoculation group: inoculation of bacterial liquid only (N=4). Paracentesis of 0.1 ml of the aqueous humor was performed, and the total inoculant volume was 0.1 ml/eye. The final concentration of bacteria in the mixture was adjusted to 10^4 CFU/eye.

Corneal opacity, ciliary injection, hypopyon and discharge were assessed. Eyes with three or more of these conditions were classified as endophthalmitic. The aqueous humor (0.05 ml/eye) was cultured 24 or 48 hours after inoculation, after general anesthesia. The rabbit was euthanized 48 hours after

inoculation and ophthalmectomy was performed. After general anesthesia, 4 ml of thiopental sodium (25 mg/ml) was injected into the cardiac sac as euthanasia. A culture of the vitreous was then made. The specimens were scanned under scanning electron microscope (Japan Electron Optics Laboratory JSM-5410) at 15 kV.

Bonferroni's method was used for statistical analyses of the difference detection of the population rate of a multi crowd. The significance level was set to 0.01.

Results

1. Development of rabbit bacterial endophthalmitis model

The endophthalmitis rate is shown in Table 1. In the 10^7 CFU/eye inoculation group, endophthalmitis rate at 6 hours after inoculation was calculated and continued thereafter for 72 hours after inoculation. In the 10^3 CFU/eye inoculation group, there were no cases of endophthalmitis during the observation period. The vitreous cultures were positive in all cases of endophthalmitis.

The positive aqueous humor culture rate is shown in Table 2. Aqueous humor cultures in the 10^3 CFU/eye inoculation group were negative after 6 hours and remained negative thereafter. Aqueous humor cultures in the 10^7 CFU / eye inoculation group were positive until 24 hours after inoculation. Thereafter, all aqueous humor cultures were negative, regardless of the severity of endophthalmitis signs. Endophthalmitis findings were examined in histopathologic specimens according to inoculation bacteria count. Edema of the cornea, vasodilation of iris stroma vessel, trabeculum abscess, hypopyon, and bacterial vitreous invasion were observed. Bacterial colonization of the iris was observed in a specimen with a negative aqueous humor culture in the 10^7 CFU/eye inoculation group (Photo 1).

2. Effects of viscoelastic materials on bacterial endophthalmitis

The endophthalmitis rate is shown in Table 3. In the Mixed inoculation group at 100 CFU/eye, the endophthalmitis rate was 8 of 8 eyes and 7 of 8 eyes with Healon and Viscoat, respectively. No endophthalmitis occurred at 100 CFU/eye in the Separate inoculation group or the Bacteria inoculation group. A significant difference in endophthalmitis rate was observed between the Mixed inoculation group and the Separate inoculation group ($p<0.01$, Bonferroni's method). No significant difference in the bacterial endophthalmitis rate was observed between Healon

Table 1 Endophthalmitis rate after inoculation

	10^3 CFU/eye					10^7 CFU/eye				
Observation time after inoculation (Hours)	6	12	24	48	72	6	12	24	48	72
Total eyes	10	8	6	4	2	10	8	6	4	2
Endophthalmitis eyes	0	0	0	0	0	10	8	6	4	2
Crisis (%)	0	0	0	0	0	100	100	100	100	100

Endophthalmitis was present in the 10^7 CFU/eye inoculation group 6 hours after inoculation, and continued thereafter. Endophthalmitis did not develop in the 10^3 CFU/eye inoculation group. All vitreous cultures were positive in cases of endophthalmitis.

Table 2 Rate of positive aqueous humor cultures

	10^3 CFU/eye					10^7 CFU/eye				
Culture time after inoculation (Hours)	6	12	24	48	72	6	12	24	48	72
Total eyes	2	2	2	2	2	2	2	2	2	2
Culture positive eyes	0	0	0	0	0	2	2	2	0	0
Mean CFU (CFU/0.05ml)						1000	500	380		
Culture positive rate (%)	0	0	0	0	0	100	100	100	0	0

All aqueous humor cultures of the 10^3 CFU/eye inoculation group were negative. Aqueous humor culture of the 10^7 CFU/eye inoculation group were positive until 24 hours. After that, all aqueous humor cultures were negative, regardless of the severity of endophthalmitis.

Table 3 Endophthalmitis rate after inoculation by groups

	Mixed		Separate								Bacteria
			HL				VIS				
	HL	VIS	0	6	12	24	0	6	12	24	
Total eyes	8	8	8	8	8	8	8	8	8	8	8
Endophthalmitis eyes	8	7	0	0	0	0	0	0	0	0	0
Crisis rate (%)	100	87.5	0	0	0	0	0	0	0	0	0

HL: Healon, VIS: Viscoat
There was a significant difference in endophthalmitis rate between the Mixed inoculation group and the Separate inoculation group ($p<0.01$). There was no significant difference in bacterial endophthalmitis rate between the Healon and Viscoat groups ($p>0.05$). All vitreous cultures of endophthalmitis cases were positive.

Table 4 Positive aqueous humor culture rate by groups

	Mixed				Separate				Bacteria	
	HL		VIS		HL		VIS			
Culture time after inoculation	24	48	24	48	24	48	24	48	24	48
Total eyes	8	8	8	8	32	32	32	32	8	8
Culture positive eyes	8	0	8	0	0	0	0	0	0	0
Mean CFU (CFU/0.05ml)	1245		918							
Crisis rate (%)	100	0	100	0	0	0	0	0	0	0

HL: Healon, VIS: Viscoat
Remarkable bacterial proliferation was observed at 24 hours after inoculation in the Mixed inoculation group. However, aqueous humor cultures were negative at 48 hours. Aqueous humor cultures were negative in the Separate inoculation group and the Bacteria inoculation group.

and Viscoat ($p>0.05$, Bonferroni's method). All vitreous cultures were positive in cases of endophthalmitis.

The aqueous humor culture positive rate is shown in Table 4. Remarkable bacterial proliferation was observed at 24 hours after inoculation in the Mixed inoculation group. However, aqueous humor cultures were negative at 48 hours. Aqueous humor cultures were negative in the Separate inoculation group and the Bacteria inoculation group.

Histopathologic findings of endophthalmitis were noted in the Mixed inoculation group, but not in the Separate inoculation group or the Bacteria inoculation group. After inoculation, residual viscoelastic material was observed. Bacterial proliferation in residual viscoelastic material was observed in the Mixed inoculation group (Photo 2). Limitation of polynuclear leukocyte movement in the viscoelastic material layer was noted in the Mixed inoculation group (Photo 3), i.e., leukocytes did not reach bacteria covered with the viscoelastic material. Bacterial colonization of the ciliary processes was observed in a specimen from a negative aqueous humor culture in the Mixed inoculation group (Photo 4).

3. Prevention of bacterial endophthalmitis with viscoelastic materials and newquinolone

The endophthalmitis rate at 10^4 CFU / eye is shown in Table 5. In the Antibacterial Visco group, 1 of 10 eyes treated with Healon and 2 of 10 eyes treated with Viscoat showed signs of endophthalmitis. In the Eye Drop Treatment group, 9 of 10 eyes treated with Healon and 10 of 10 eyes treated with Viscoat showed signs of endophthalmitis. All vitreous cultures were positive in cases of endophthalmitis.

A significant difference in the endophthalmitis rate was also observed with Healon between the Antibacterial Visco group and the Eye Drop Treatment group ($p<0.01$, Bonferroni's method). There was also a significant difference in endophthalmitis rate with Viscoat between the Antibacterial Visco group and the Eye Drop Treatment group ($p<0.01$, Bonferroni's method). No significant difference was

Table 5 Endophthalmitis rate after inoculation

	Antibacterial Visco		Eye Drop Treatment		Non Treatment		Bacteria
	HL	VIS	HL	VIS	HL	VIS	
Total eyes	10	10	10	10	4	4	4
Endophthalmitis eyes	1	2	9	10	4	4	0
Crisis rate (%)	10	20	90	100	100	100	0

HL: Healon, VIS: Viscoat

There was a significant difference in endophthalmitis rate with Healon between Antibacterial Visco group and Eye Drop Treatment group ($p<0.01$). There was also a significant difference in endophthalmitis rate with Viscoat between Antibacterial Visco group and Eye Drop Treatment group ($p<0.01$). There was no significant difference in endophthalmitis rate between Healon and Viscoat in any group ($p>0.05$). In Non-Treatment group, all eyes suffered endophthalmitis. There was no significant difference in endophthalmitis rate between Non-Treatment group and Eye Drop Treatment group. In Bacteria inoculation group, no eyes contracted endophthalmitis.

observed in endophthalmitis rate between Healon and Viscoat in any group ($p>0.05$, Bonferroni's method). In the Non-Treatment group, all eyes suffered endophthalmitis. No significant difference was observed in endophthalmitis rate between the Non-Treatment group and the Eye Drop Treatment group. In the Bacteria inoculation group, no eyes contracted endophthalmitis.

Aqueous humor cultures taken 24 and 48 hours after inoculation were negative, regardless of endophthalmitic status. Limited migration of polynuclear leucocytes through the viscoelastic material layer was observed (Photo 5). In the Antibacterial Visco group, limited migration of polynuclear leucocytes did not result in endophthalmitis.

Discussion

In the present study using a rabbit bacterial endophthalmitis model, bacterial endophthalmitis occurred at a concentration of 10^7 CFU/eye, but not at 10^3 CFU/eye, a finding which suggests that it is necessary to consider the invading bacterial count after intraocular surgery. Although disinfection and antibacterial drug use are painstakingly utilized to prevent postoperative bacterial endophthalmitis[23-27], many surgeons have reported bacteria spread to the aqueous humor during cataract surgery even when all reasonable preventive measures had been taken[27-36]. Fortunately, invading bacterial counts tend to be very low in recent intraocular surgery[24,30]. Moreover, when bacteria invade, the anterior chamber has stronger resistance than the vitreous. In animal experiments, bacterial endophthalmitis develops at a concentration as low as 10 CFU/eye in vitreous inoculation[37]. A higher number of bacteria are necessary in anterior chamber inoculations: 10,000 or more[38-40]. The results of the present study do not contradict these findings. Our findings suggest that the anterior chamber is an inhospitable location for bacterial proliferation.

However, the reason why the anterior chamber is so resistant to bacterial proliferation is unknown. It has been suggested that clearance activity in the aqueous humor protects against bacterial invasion. Bacteria in the anterior chamber are excluded from the trabeculum by this clearance. Moreover, phagocytes in the trabecula show remarkable phagocytic activity in the eye. A strong inflammatory reaction including ciliary injection is observed in this area in early stage endophthalmitis. Moreover, migration of phagocytes, such as neutrophilic granulocytes, from the vessel of the iris has been noted. Needless to say, antibacterial drug action at surgery might also be important. In the present study, aqueous humor cultures remained negative despite inoculation of bacteria to the anterior chamber and the presence of severe endophthalmitis. Furthermore, bacterial colonization of the iris was observed in a negative aqueous humor culture group. Bacteria in the anterior chamber are not easily detected in an investigation of the aqueous humor. There are many reports suggesting that, in cases of postoperative bacterial endophthalmitis, vitreous cultures are superior to aqueous humor cultures. The results of the present study confirm these reports[5,41]. Our findings

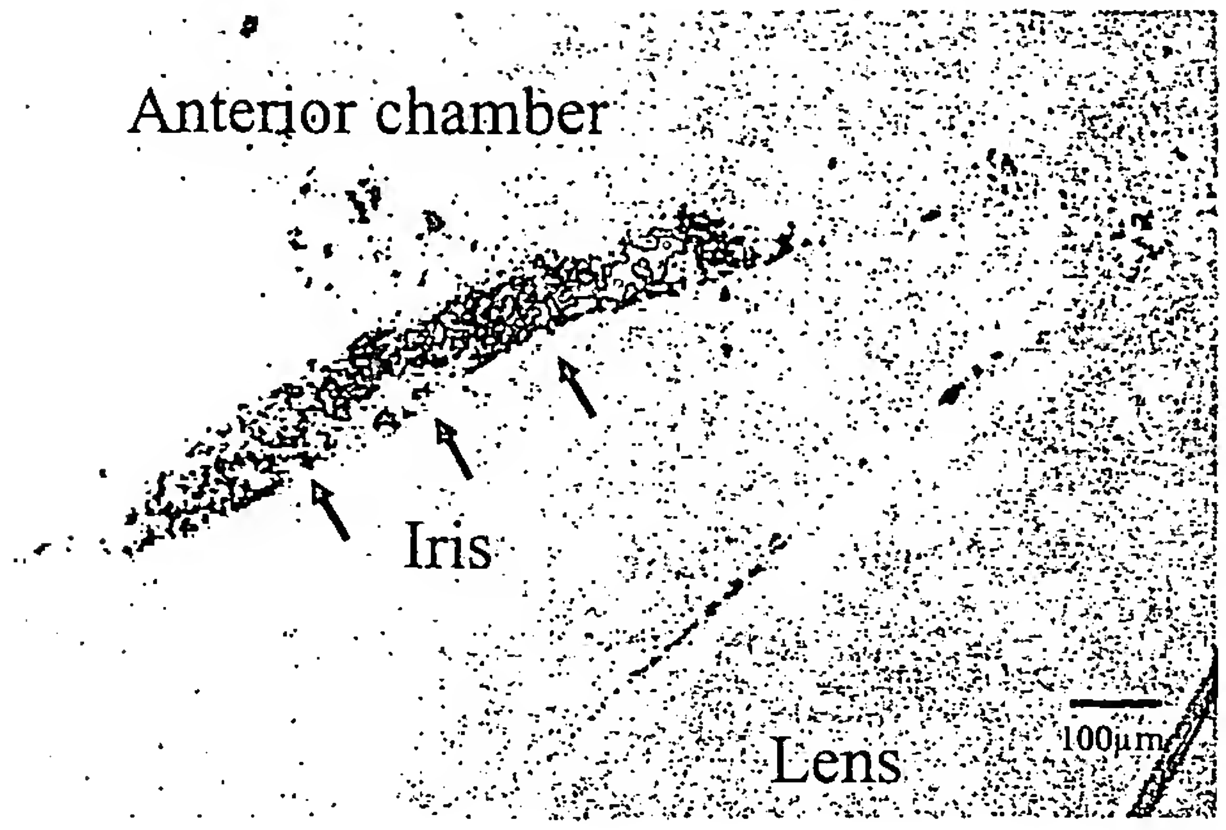
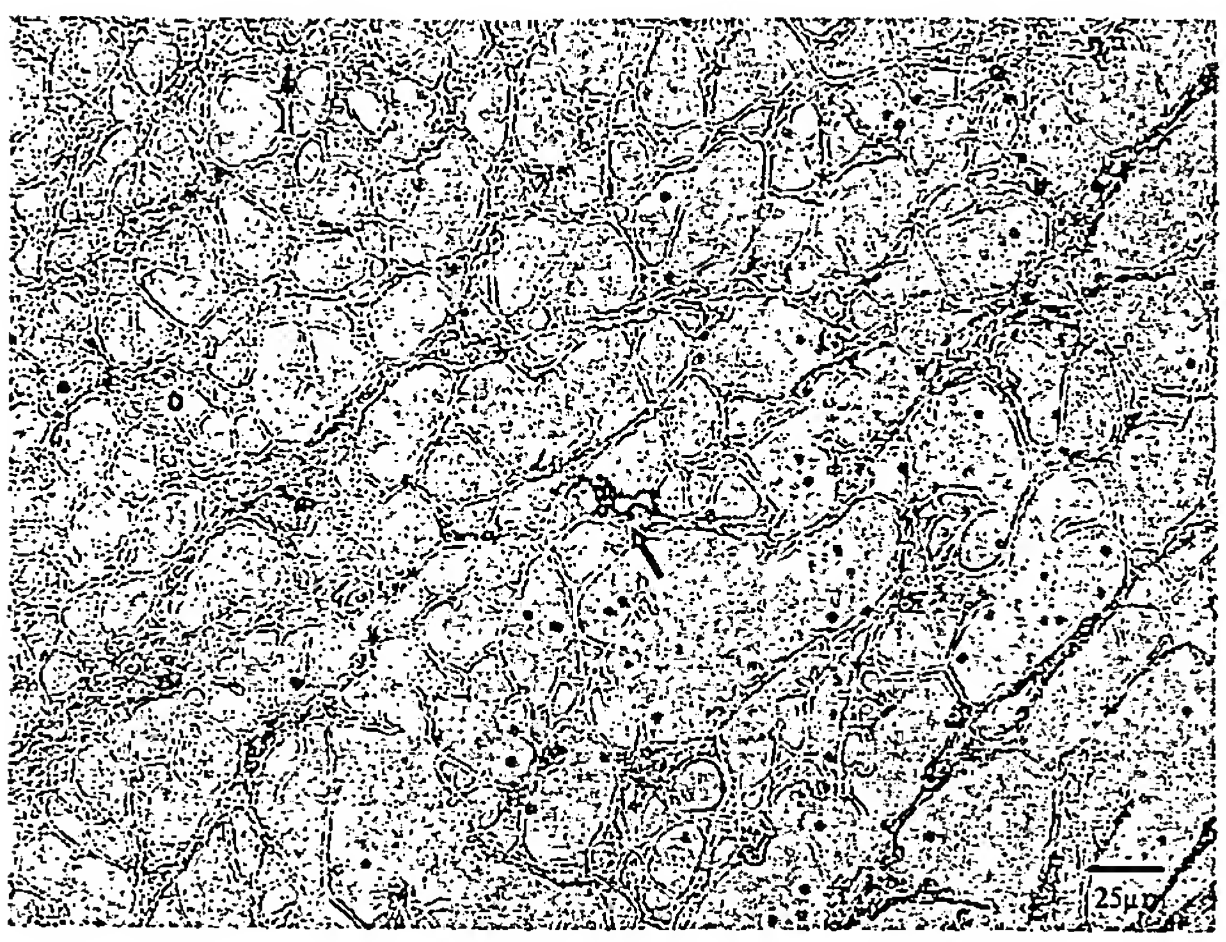


Photo 1



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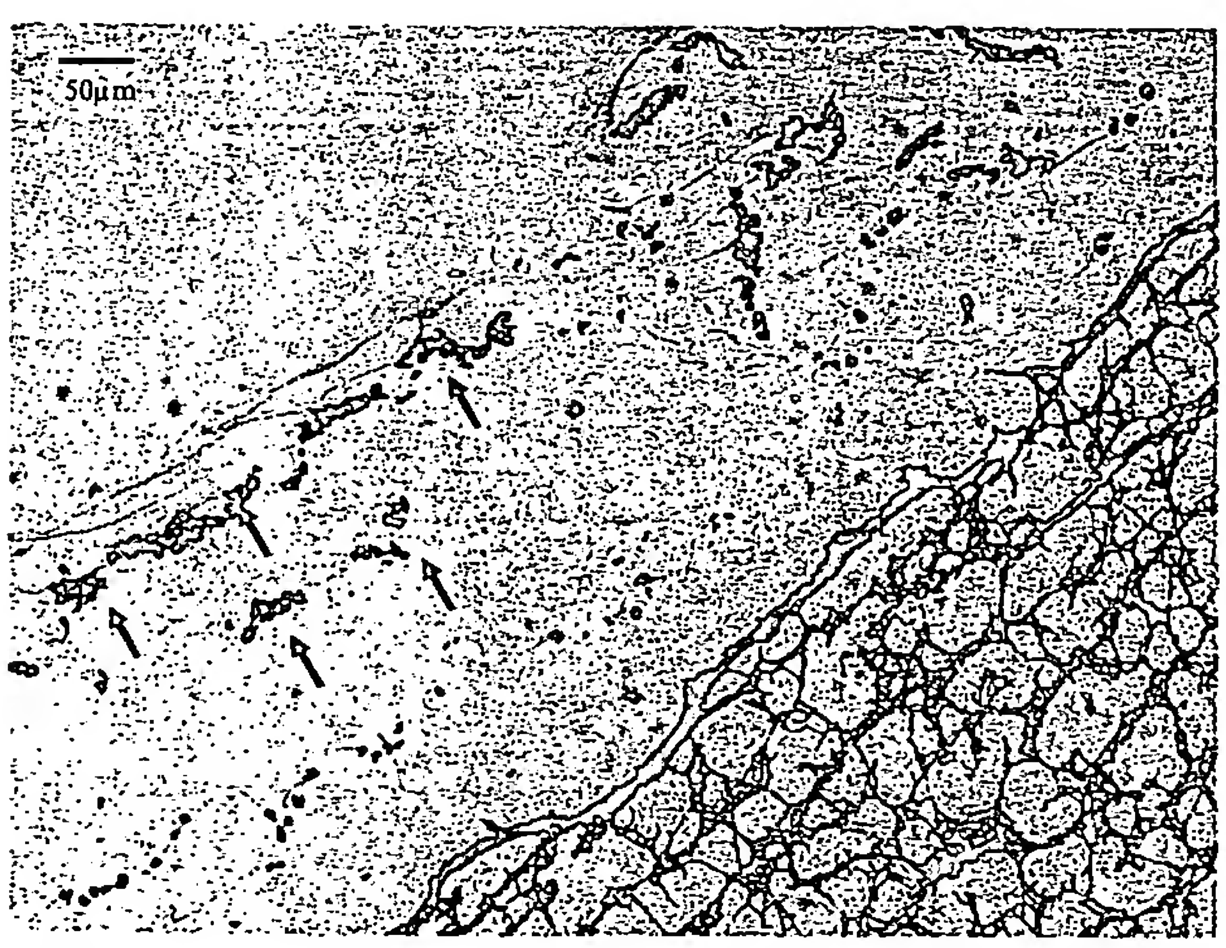

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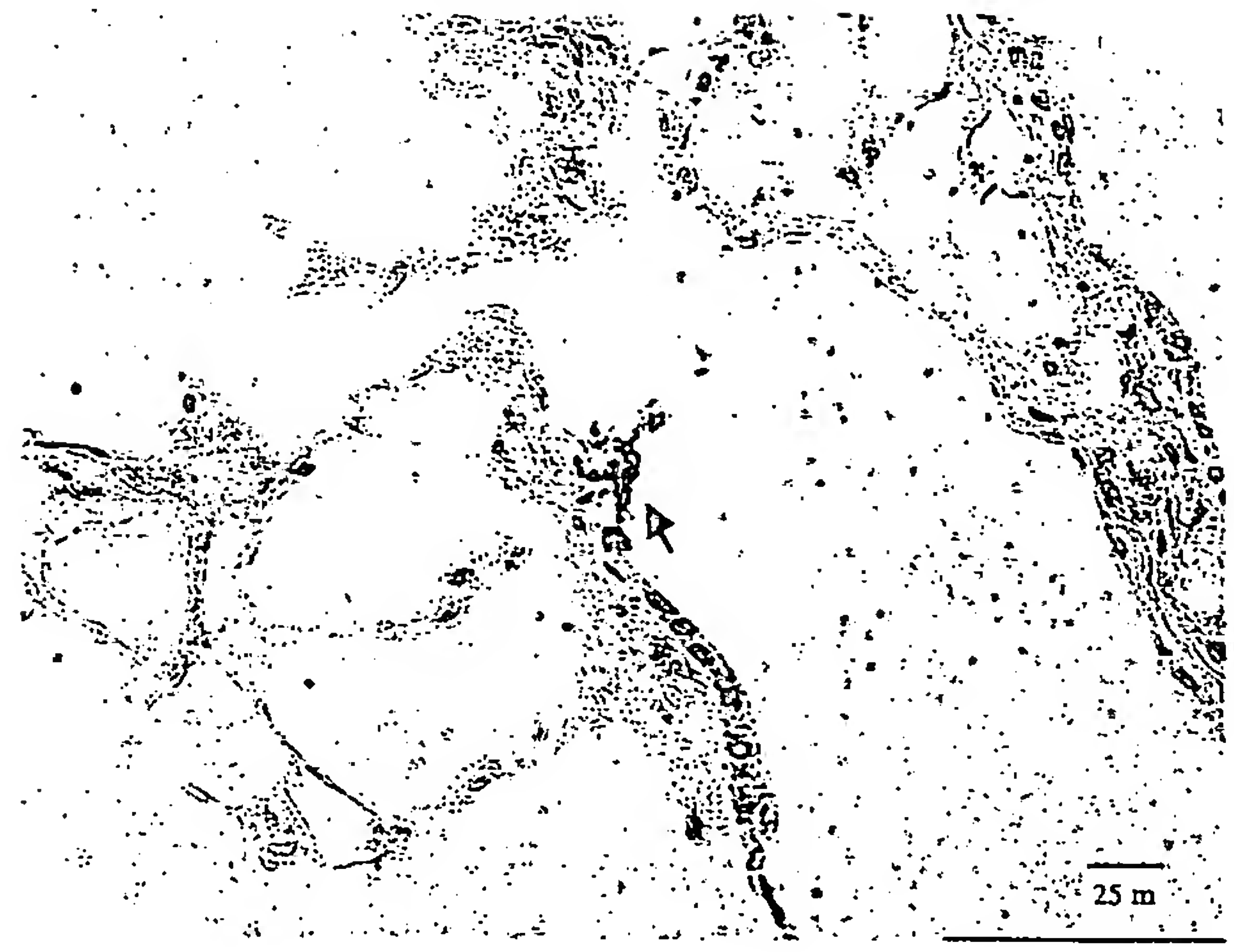

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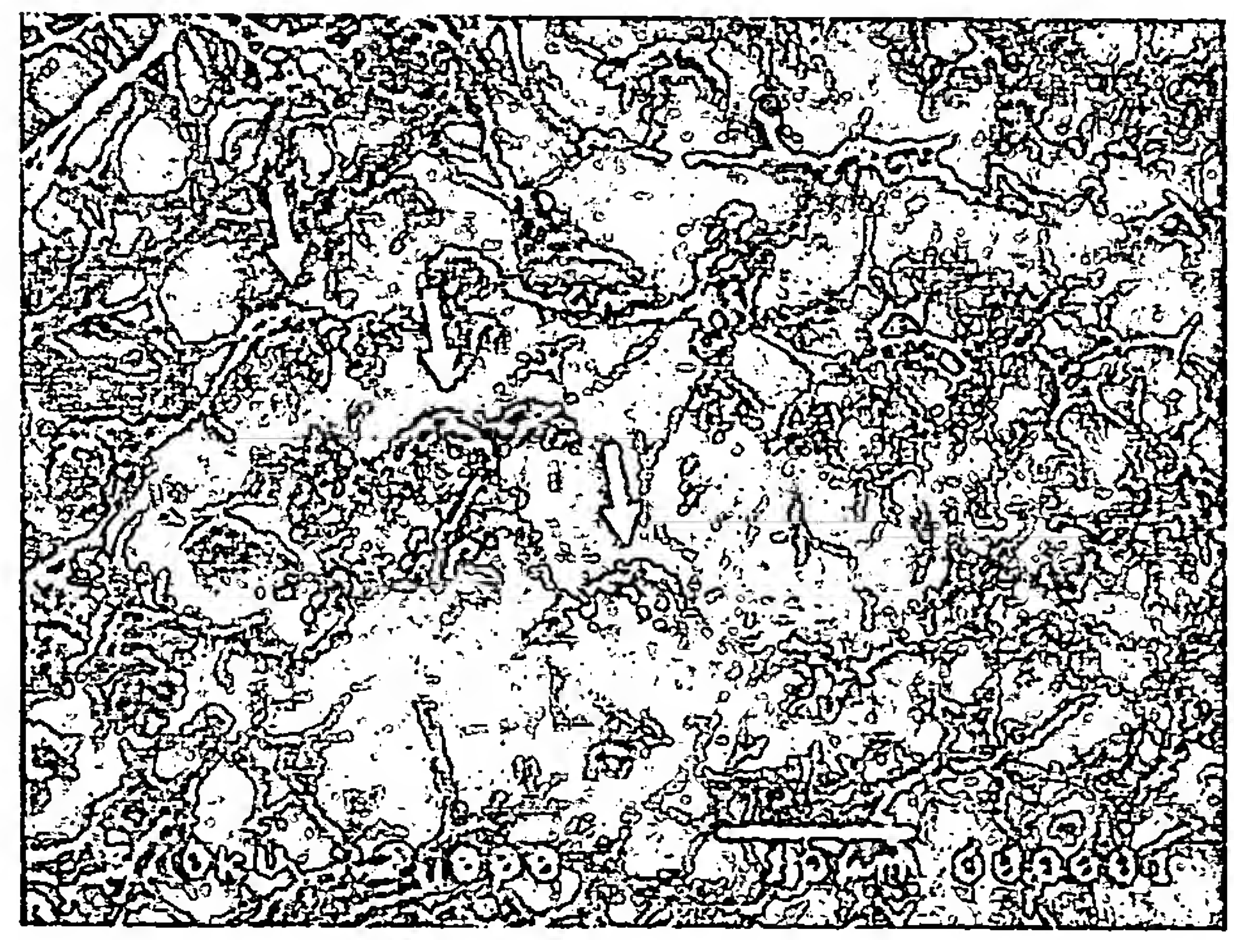

Photo 5

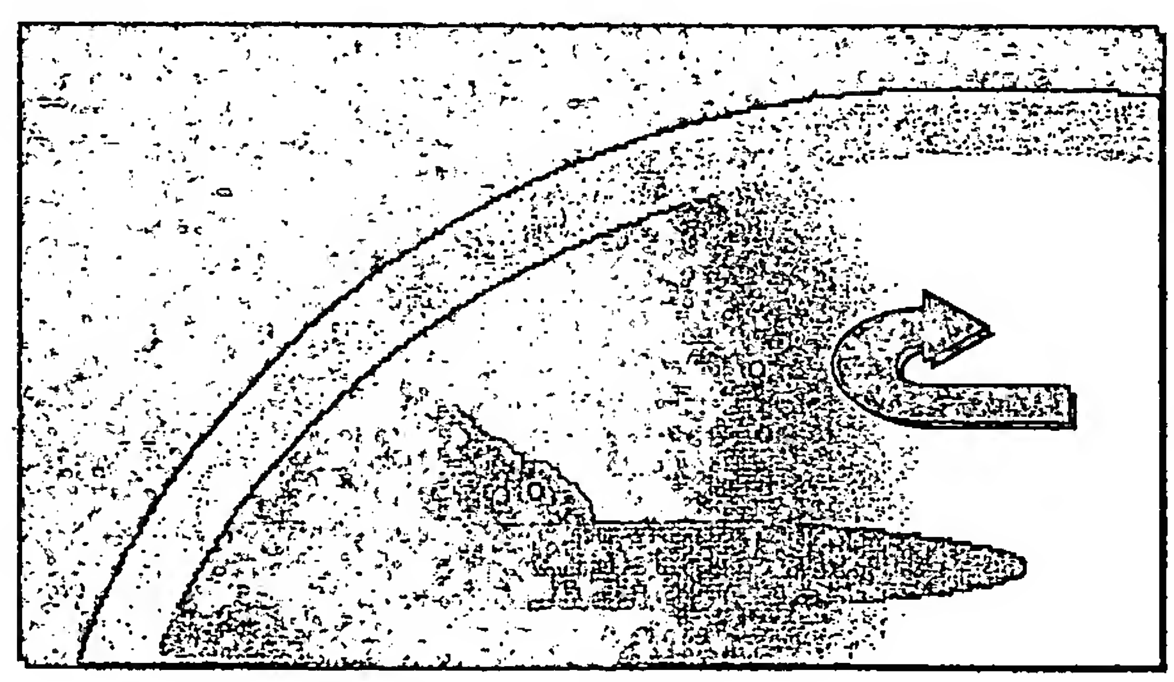

Fig. 1

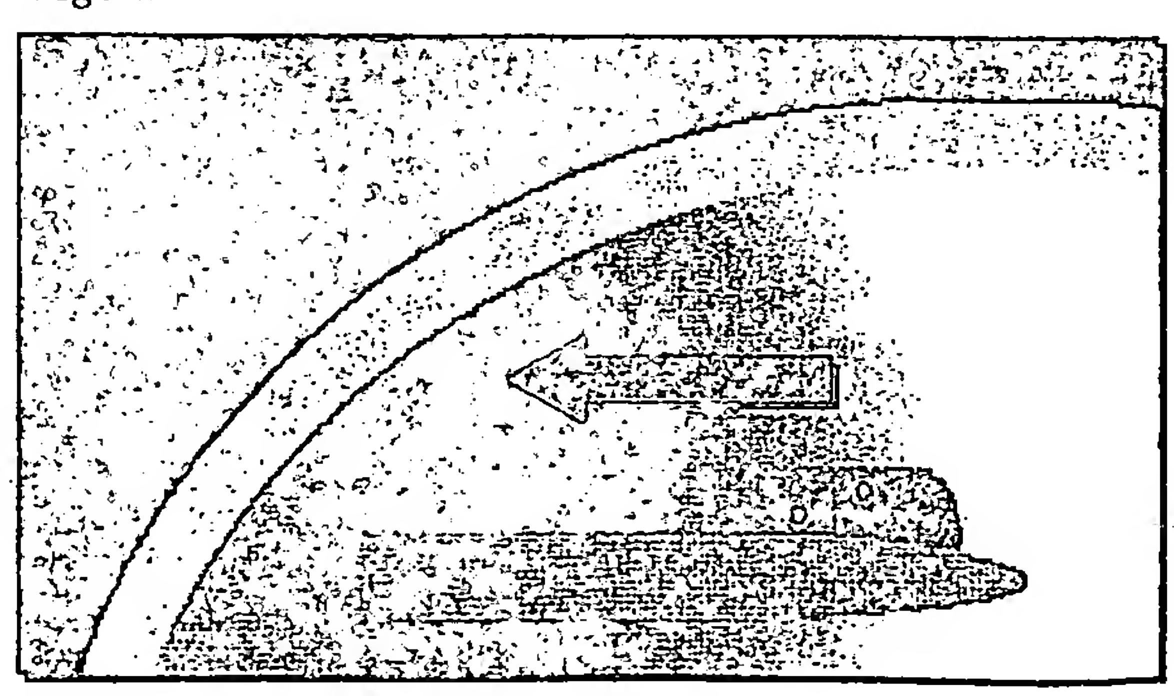

Fig. 2

suggest that through clearance and phagocytosis, the aqueous humor is capable of excluding even large numbers of bacteria. Because of clearance and phagocytosis, the bacterial count decreased with time and negative cultures were obtained after 48 hours in eyes that had been endophthalmitis.

Postoperative bacterial endophthalmitis includes anterior and posterior postoperative bacterial endophthalmitis. Anterior endophthalmitis remains in the anterior chamber, and posterior endophthalmitis reaches the vitreous. Posterior endophthalmitis is refractory, even if emergency vitreous surgery is performed, and visual acuity outcomes are poor. The entry point for bacteria in anterior intraocular surgery such as cataract surgery is the anterior chamber[28]. In modern cataract surgery, the possibility of bacteria immediately reaching the vitreous is very low when rupture of the posterior capsule does not occur[3,5,6,38,39,42]. In addition, the number of bacteria invading the anterior chamber is low[30]. However, postoperative bacterial endophthalmitic crises can still occur. The mystery is why a small number of bacteria is capable of evading the robust defenses of the anterior chamber to cause bacterial endophthalmitis.

In the present study of the effects of viscoelastic materials on bacterial endophthalmitis, endophthalmitis occurred at a concentration of 100 CFU/eye in the Mixed inoculation group. Endophthalmitis in the anterior chamber inoculations does not usually develop at this concentration, which suggests that the presence of viscoelastic materials contributes to the incidence of endophthalmitis. However, because endophthalmitis did not develop in the Separate inoculation group in the present study, it appears that viscoelastic materials alone do not make endophthalmitis. There is one report of endophthalmitis due to contaminated viscoelastic material[43]. Residual viscoelastic material in intraocular surgery caused angle occlusion and increased intraocular pressure[44,45]. Moreover, residual viscoelastic material limits aqueous humor clearance and cause pooling of bacteria by angle occlusion (Fig. 1). In addition, it might shield bacteria from aqueous humor clearance and phagocytosis (Fig. 2). Angle occlusion due to viscoelastic material was present in both the Mixed inoculation group and the Separate inoculation group. However, the protection of bacteria by viscoelastic material in the Mixed inoculation group was more obvious than in the Separate inoculation group; endophthalmitis was indeed observed in the Mixed inoculation group in the present study. Regarding the reason for the occurrence of endophthalmitis with viscoelastic material, the protection of bacteria, rather than angle occlusion, appears more likely. Bacterial proliferation occurs under the viscoelastic material layers.

There was no significant difference in the endophthalmitis rate between Healon and Viscoat in this study. Viscoat, a dispersive viscoelastic material,

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Photo 1 Image of a rabbit eye infected with methicillin-resistant *Staphylococcus aureus* MK99-3, 72 hours after inoculation. (Giemsa stain, ×100)
Bacterial colonization of the iris (arrow) in a specimen from a negative aqueous humor culture from the 10^7 CFU/eye inoculation group.

Photo 2 Image of a rabbit eye infected with methicillin-resistant *Staphylococcus aureus* MK99-3, 48 hours after inoculation with Viscoat. (Giemsa stain, ×400)
Bacterial proliferation (arrow) can be seen in residual viscoelastic material.

Photo 3 Image of a rabbit eye infected with methicillin-resistant *Staphylococcus aureus* MK99-3, 48 hours after inoculation with Viscoat. (Hematoxylin-Eosin stain, ×200)
Interruption of polynuclear leukocyte migration (arrow) by viscoelastic material layer occured in the Mixed inoculation group.

Photo 4 Image of a rabbit eye infected with methicillin-resistant *Staphylococcus aureus* MK99-3, 48 hours after inoculation with Healon. (Giemsa stain, ×400)
Bacterial colonization (arrow) on ciliary processes can be seen in a negative aqueous humor culture from the Mixed inoculation group.

Photo 5 Electron microscopic image of a rabbit eye infected with *Staphylococcus aureus* Smith, 48 hours after the inoculation (×2000).
This image of the iris surface was taken from the corneal side. Retarded migration of polynuclear leucocytes (arrow) through the viscoelastic material layer (net shape) can be seen.

Fig. 1 Residual viscoelastic material can cause pooling of bacteria by angle occlusion

Fig. 2 Residual viscoelastic material might protect bacteria by shielding them from the clearance of aqueous humor flow and phagocytosis.

more readily remains in the intraocular space[16-18], because of its rheological characteristics. Thus it is possible that Viscoat use may result in some degree of endophthalmitis. However, the aspiration procedure usually performed during surgery was not done in the present study. Thus, residue of both viscoelastic materials remained in the anterior chamber in the present study, which may explain the lack of a significant difference between Healon and Viscoat.

In this study, a realistic bacterial count of 100 CFU/eye was inoculated. Other studies of endophthalmitis used anterior chamber inoculations with an unrealistic bacterial count of 10^5 CFU/eye[38-40]. In a recent report, the bacterial count in an aqueous humor culture immediately after cataract surgery corresponded to the bacterial count in the present study[24,30], suggesting that the possibility of endophthalmitis with a bacterial count of 100 CFU/eye is realistic. In light of these findings, the necessity of viscoelastic material removal becomes clear. However, because complete removal of residual viscoelastic material is impossible, precaution against endophthalmitis appears necessary.

In the present study of prevention of bacterial endophthalmitis with viscoelastic materials in combination with newquinolones, LVFX eye drop treatment was ineffective in preventing endophthalmitis in the presence of viscoelastic material. Although *Staphylococcus aureus* Smith is an LVFX-sensitive strain, LVFX penetration might have been delayed by the presence of viscoelastic material. Indeed, a delay in antibacterial drug penetration by viscoelastic material was reported in the previous article. Because of this delay, the antibacterial drug was washed out by aqueous flow before LVFX penetrated the viscoelastic material. No difference between Healon and Viscoat was observed in the present study because the anterior chamber washing during the actual cataract surgery was not performed in the present study.

Our findings clearly show that Antibacterial Visco, a mixture of viscoelastic material and antibacterial drug, prevented endophthalmitis. In the previous research, we showed that antibacterial drug penetration is not decreased by admixture with viscoelastic material and that viscoelastic material combined with LVFX at AQCmax concentration can prevent bacterial endophthalmitis.

These studies have shown that a small number of bacteria can cause bacterial endophthalmitis in the presence of viscoelastic material, and that the use of viscoelastic material makes antibacterial eye drop treatment ineffective. However, the preventive effect of Antibacterial Visco was demonstrated. Postoperative bacterial endophthalmitis is a very serious complication[1,2], and a number of possible risk factors have been proposed, including posterior capsular rupture, diabetes mellitus, contamination of drugs or surgical instruments, or insufficient disinfection[19,46-49]. We investigated the presence of residual viscoelastic material as a risk factor. The followings are tentative explanation for the acceleration of endophthalmitis by viscoelastic materials:

1) Sheltering of bacteria from the clearance effect of aqueous humor by viscoelastic material.

2) Delay in antibacterial drug penetration by viscoelastic material.

These are the negative consequences of viscoelastic material use. However, we believe that such materials can instead be used to prevent endophthalmitis. Antibacterial Visco is an attempt to facilitate antibacterial drug delivery in cases where viscoelastic materials are necessary. Indeed, other applications have already been investigated: anesthetic viscoelastic materials have been reported[50-52], as have viscoelastic materials with gentamicin for bacterial endophthalmitis treatment[53,54].

The use of viscoelastic material remains indispensable to modern intraocular surgery. In particular, viscoelastic materials are important in cataract surgeries, which represent the majority of intraocular surgeries[13,14]. Further development of viscoelastic materials is required. One such development was the addition of endothelium protection to the essential space maintenance ability of viscoelastic material [18-21]. The intraocular is not sterile during surgery and the viscoelastic material has not been completely removed at the end of operation. Bacterial endophthalmitis can easily occur in the presence of residual viscoelastic material. In addition, endophthalmitis in the presence of viscoelastic material is refractory to eye drop treatment. In conclusion, our findings show that mixture of antibacterial drug and viscoelastic material can prevent endophthalmitis.

In addition, we believe that development of Antibacterial Visco would be a further improvement. We have already developed a "double protection" viscoelastic material by adding antibacterial action to a viscoelastic which is also capable of protecting the corneal endothelium. We hope that this new viscoelastic material can improve the safety of future intraocular surgical procedures.

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粘弾性物質による細菌性眼内炎への影響および ニューキノロン含有粘弾性物質による 細菌性眼内炎予防

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要約

目的：前回の発表で粘弾性物質によるニューキノロン薬の薬剤移行の阻害を報告した。今回われわれは，粘弾性物質による細菌性眼内炎への影響および粘弾性物質とレボフロキサシンの合剤による細菌性眼内炎予防について検討した。

方法：1）MRSA を白色家兎の前房に投与し眼内炎モデルを作成した。2）粘弾性物質の影響を調べるために以下の3群を比較した。A）粘弾性物質と MRSA を混合し投与した群。B）粘弾性物質投与後に MRSA を投与した群。C）細菌のみ投与した群。3）粘弾性物質とニューキノロン薬の合剤の効果を調べるために以下の4群を比較した。A）抗菌薬含有粘弾性物質使用群，B）点眼加療群，C）粘弾性物質使用群，D）細菌のみ投与した群。

結果：1）10^7 CFU/eye において細菌性眼内炎が作成できたが，10^3 CFU/eye では作成できなかった。2）10^3 CFU/eye の低濃度投与でも粘弾性物質混合群では眼内炎が発生した。混合しなかった群では眼内炎が発生しなかった。3）抗菌薬含有粘弾性物質では有意に眼内炎が予防できた。しかし，抗菌薬点眼では治療困難であった。

結論：粘弾性物質は細菌性眼内炎を誘発した。抗菌薬含有粘弾性物質は細菌性眼内炎予防に有効である。

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後藤　憲仁（獨協医大）

252 家兎眼水晶体・硝子体切除後のマイトマイシンC塗布の有無による残存前嚢混濁の比較

宮本　　武（和歌山県医大）

れを *E. coli* 塗
【結果】①24時
察された。②24
円の形成がみら
【結論】水溶性
れた。H60M は
いると考えられ

248　抗菌薬含有粘弾性物質の術後細菌性眼内炎予防としての可能性

○田中康一郎、小早川信一郎、岡島行伸、片山康弘、忍田拓哉、
杤久保哲男（東邦大学第一眼科）
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阪西弘太郎（メニコン）

<目的>我々は、前回の日眼総会にて残留粘弾性物質による細菌性眼内炎の危険性を示した。そこで、抗菌薬を含有する粘弾性物質の術後細菌性眼内炎への予防効果を検討した。
<方法>日本白色家兎（16羽32眼）を用いて細菌性眼内炎モデルを作成した。菌種は*Staphylococcus aureus*（Smith株）、抗菌薬はレボフロキサシン（LVFX）、粘弾性物質はHealon及びViscoatを用いた。家兎前房に菌液を接種し、24、48時間後に観察及び前房水培養、接種48時間後に眼球を摘出し、病理検索を行った。接種方法は、１）抗菌薬含有粘弾性物質群（粘弾性物質＋菌液＋抗菌薬）、２）点眼治療群（粘弾性物質＋菌液）とした。両群とも接種菌量は10^4cfu／eye、粘弾性物質の抗菌薬濃度はLVFX4μg／ml、接種総量は0.1ml／eyeとした。点眼治療群では接種前日から眼球摘出時まで4回／日の0.5%LVFX点眼を行った。
<結果>　眼内炎の発症は、抗菌薬を含有する粘弾性物質群では、ヒーロン(1／8)、ビスコート(2／8)であり16眼中3眼にみられた。点眼治療群では、ヒーロン(7／8)、ビスコート(8／8)であり、16眼中15眼が発症した。両群間において有意差が見られた（$p<0.01$）。24及び48時間後の前房水培養は陰性であった。眼内炎の発症眼から細菌付着像が観察された。
<結論>抗菌薬含有粘弾性物質は家兎眼内炎モデルにおいて細菌性眼内炎の発症率を有意に低下させた。抗菌薬含有粘弾性物質は術後細菌性眼内炎対策として大いなる効果が期待できる。

250　ド
抑制

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吉田登茂子[1]、
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目的：ハイドロジ
して応用し、薬物
方法：生後８週の
を採取し、培養液
5%CO_2)。培養細
使用して細胞密度
て親水性眼内レン
水、ジクロフェナ
チン、フルオロウ
1時間浸透させた眼
ルチャーインサー
30,000個培養水晶体
目に10%ホルマリ
リン・エオジン染
性状を観察、scion
た。
結果：対照群では
細胞伸展がみられ
ゲン膜上の細胞付
比較するとコルヒ
の順に抑制効果が
結論：ハイドロジ
in vitro で後発白内